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Effects of Phenylmercuric Acetate on the Transpiration and Physiology of Quaking Aspen

Kenneth O. Higginbotham

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THE EFFECTS OF PHENYLMERCURIC ACETATE ON THE TRANSPIRATION AND PHYSIOLOGY OF QUAKING ASPEN

by

Kenneth O. Higginbotham

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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Approved:

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Committee Member

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Kenneth O. Higginbotham

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ABSTRACT

The Effects of Phenylmercuric Acetate on
the Transpiration and Physiology
of Quaking Aspen

by

Kenneth O. Higginbotham, Master of Science

Utah State University, 1970

Major Professor: Dr. John D. Schultz
Department: Forest Science

The effects of phenylmercuric acetate, a plant anti-transpirant, on reducing transpiration in aspen were studied in northern Utah.

A significant difference in sap flow rates of treated and untreated trees was noticed on several days during the growing season. A numerical difference was always present. The size of leaf stomate opening also responded in the same manner.

Annual ring growth was significantly reduced by the treatment in 1968 but not in 1967. The production of total available carbohydrates was not reduced significantly by the treatment.

A multiple regression equation to predict sap velocity of untreated trees using climatic variables was developed. A correlation coefficient of 0.55 was obtained.

(55 pages)

INTRODUCTION

Since 1911 when the first experimental watershed in the United States was established at Wagon Wheel Gap, Colorado, scientists have attempted to increase the yield of water from forested watersheds by manipulating vegetation. Plants transpire large amounts of water into the atmosphere and it has been generally believed that reductions of this water loss might increase water yield in the form of streamflow. Practices employed have usually involved removing, killing, or defoliating trees on watersheds.

Currently, some wildland hydrologists are attempting to reduce water use and to increase water yield by using anti-transpirant chemicals. If one of these chemicals can be found that will reduce water loss without damaging trees or greatly reducing their growth, then water yield increases could be accomplished without impairing aesthetic values or increasing sediment yield. These two problems frequently accompany other types of treatments.

Quaking aspen (*Populus tremuloides* Michx.) covers thousands of acres of mountainous country in the Intermountain Region of the western United States. Its economic value is limited although it can be used in the manufacture of paper pulp. Water for processing pulp, however, is very limited in the region. The species is thought to transpire great quantities of water into the atmosphere where it is of no direct use to man. Aspen does constitute a valuable protective ground cover though and as such it is desirable not to remove it in many instances.

In this study, an attempt was made to reduce the transpiration of aspen using an anti-transpirant chemical. The one chosen was phenylmercuric acetate, subsequently referred to simply as PMA. Its effects on transpiration and growth of quaking aspen are reported here.

Objectives

The main objective of this thesis is to describe and explain the effects of phenylmercuric acetate on the transpiration, production of carbohydrates, and diameter growth of quaking aspen in a natural environment. A secondary objective is to predict the transpiration rate of untreated quaking aspen trees using meteorological parameters that can be measured with readily available instruments.

REVIEW OF LITERATURE

Mechanisms of Transpiration

Ascent of water

The movement of water from soil into the roots of trees is a diffusion process. Water moves from an area of high total soil water potential to an area of lower potential. It moves from cell to cell until it reaches the vascular system of the root. The xylary cells (vessels and/or tracheids) functioning in the ascent of sap are dead cells. According to Zimmerman (1963), water movement in these cells is in columns which are held together by cohesive and adhesive forces. Other theories have been proposed to account for flow to the top of a 300-foot tree. Kozlowski (1964) stated that 20 atmospheres of tension are required to lift water to a height of 300 feet and that water columns in vessels have enough tensile strength to withstand this pressure without breaking. He also stated, with respect to aspen, that water transport takes place in the outer few annual growth rings. Sap ascends in an interlocking zig-zag pattern in this species.

When water reaches the leaf, it passes from cell to cell through the leaf mesophyll and finally diffuses out into the atmosphere through the leaf stomata. More than 90 percent of the water loss of a tree is through these stomata; the remaining few percent is due to cuticular transpiration.

Mechanisms of stomate opening

Zelitch (1967) stated that interacting factors cause the opening and closing of stomata. Among these are solar radiation, carbon dioxide (CO_2), and products of photosynthesis. It is well known that light is an important factor in the photosynthetic process. The guard cells that surround the stoma contain chloroplasts so they must carry on photosynthesis. CO_2 is an important ingredient in photosynthesis, and it has been shown that relatively high concentrations (0.1 - 0.2%) inhibit stomate opening. Normal concentration of CO_2 in the air is only 0.03 percent. Pallas (1966) stated that in the presence of light, glycolate, an early product of photosynthesis, decreases the osmotic potential and the total water potential in the guard cells. Water moves into the cell and the cytoplasmic system exerts a pressure on the walls. The walls are differentially thickened and so a pore is formed between them due to this pressure. To sum up then, it is the interaction of light, production of glycolate, and the concentration of CO_2 affecting the production of glycolate which determines whether a stomate will be opened or closed.

Another more plausible theory was discussed by Zelitch (1965). He explained that it is the consumption of CO_2 in the leaf mesophyll and not in the guard cell that leads to stomate opening. The low CO_2 concentration in the mesophyll leads to reduced CO_2 in the guard cells. The pH of the cells increases due to a reduction in organic acids. Starch is changed to sugar due to the increased basicity of the cells. This reduces the water potential in the guard cells and water moves into them causing opening of the stomata.

Phenylmercuric Acetate

Phenylmercuric acetate (PMA), according to Waggoner and Bravdo (1967), has been used for 25 years as an eradicant of scab fungus on apple leaves. Waggoner and Zelitch (1965) stated that PMA reacts with the sulphydryl groups in the membranes of the guard cells and reduces their permeability. Zelitch and Waggoner reported earlier (1962) that the effect of the spray is shown only on the treated surface. PMA was not even translocated from one side of the leaf to the other in their study. The effect lasted for at least 14 days on tobacco and maize plants. They also stated that PMA should be applied to the guard cells and as few others as possible to insure that photosynthesis will be as high as possible.

Photosynthesis will already be hindered by increased resistance to CO_2 diffusion due to the treatment of the guard cells.

Reduction in transpiration and photosynthesis

Zelitch and Waggoner (1962) reported that transpiration is reduced relatively more than photosynthesis by treatment with PMA. Slatyer and Bierhuizen (1964) explained why this is so. Water vapor encounters two basic resistances as it diffuses out of the stomate. These are leaf resistance and boundary layer resistance (resistance of the air). This can be expressed by the formula $T = \frac{C}{r_a+r_l}$ where:

T = resistance to diffusion out of the leaf
C = concentration of water outside the leaf
r_a = resistance of the air
r_l = resistance of the leaf

Resistance to CO_2 diffusion may be expressed as $P = \frac{C}{r_a+r_l+r_m}$ where:

P = CO_2 diffusion
r_m = resistance of the mesophyll

The term r_m is several times as large as $r_a + r_l$. Because of this and because r_a is relatively low under even moderate wind speeds, it is apparent that factors that may increase r_l may cause $r_a + r_l$ to increase several times and cause a decrease in transpiration relatively more than in photosynthesis. The PMA increases r_l but does not affect r_m. Waggoner and Zelitch (1965) stated that improved hydration of leaves with decreased transpiration may actually increase photosynthesis in some species.

Concentration of application

Davenport (1967) studied the effect that a concentration of 1 X 10^{-3} M PMA had on grasses, and he reported a slight yellowing of the leaves three days after spraying. Zelitch and Waggoner (1962) found in their studies of tobacco and maize that a concentration of 1 X 10^{-4} M gave the best results without harming the leaves of the plants. Granger and Edgerton (1966) used PMA at a concentration of 1000 ppm to close the stomata of apple leaves. The chemical damaged the leaves to a variable extent. Injury started at the distal end and moved towards the petiole.

Effect on growth

Shimshi (1963) reported that growth of sunflowers was reduced 7 percent by PMA 15 days after treatment. Davenport (1967) reported a 70 percent growth reduction in grass at a treatment concentration of 1 X 10^{-3} M. At a concentration of 1 X $10^{-3.2}$ growth was reduced 33 percent. Waggoner and Bravdo (1967) found that stem growth of red pine was reduced 15 percent by treatment with PMA at a concentration of 300 ppm. Waggoner (1967), in a study of the effects of PMA on jack pine grown in cans, reported that unsprayed trees grew

36 percent and sprayed trees 32 percent taller in one year. This difference was not significant. The trees were then cut and weighed. Stems of the sprayed trees weighed a significant 24 percent less than the untreated ones. Turner and Waggoner (1968) stated that PMA caused a 40 percent reduction in radial growth of red pine in the first year of treatment and a 32 percent reduction in the second year.

Effect of PMA on stomate opening

Hart, Schultz, and Coltharp (1969) reported that samples of aspen leaves sprayed with PMA and then fixed in a formalin acetic alcohol solution had an average stomate width of 2.4 microns. Stomate width in untreated leaves averaged 4.0 microns. This indicates that the treatment was at least partially successful although only 40 percent closure was obtained.

Methods of Measuring Transpiration

Weighing and humidity methods

Swanson and Lee (1966) discussed several methods by which transpiration or an index to transpiration can be measured. The polyethylene tent method requires air to be blown through a tent built around a plant and the difference in humidity of the air between entrance and exit can be used as an indication of the amount of transpiration. Weighing sealed, potted plants at intervals is another method described. The difference in weight over time of excised plant parts is still another method of determining the amount of water use by a plant.

Heat pulse velocity method

Because the heat pulse velocity method was used in the study reported here it will be discussed more completely at this point. According to Marshall (1958a), heat pulse velocity is an adequate measure of transpiration if only relative sap flow rates (a transpiration index) are required.

The only injury to the tree is the drilling of fine holes. Skau and Swanson (1963) explained the method in detail. Three small holes are drilled into the outer xylem tissue and a heat source and two thermocouples are inserted into these holes. The method requires the injection of heat into the tree through the heat source. The time required for the heat to move a certain distance up the tree through the sap stream and the stationary wood is measured. The instrument used is stable from -50C to +80C, and the effective diameter of velocity measurement is two centimeters. Marshall (1958b) stated that experiments with dyes have shown that dye travels up to four times faster than the heat pulse velocity in *Pinus radiata*. This is because the heat pulse is moving by conduction through cellulose as well as by convection in the moving sap. He said that hardwoods, with their coarse xylem, should retard the heat pulse to a speed less than that of the sap.

Closs (1958) gives the mathematical means by which the heat pulse velocity may be calculated. Since moving sap is not the only medium carrying the heat, the general formula $V = \frac{X}{T}$, where X is the distance traveled and T is the time in seconds, does not apply. The derived formula then is

$$V = \frac{3600(X_1 - X_2)}{2T}$$ where:

V = heat pulse velocity in cm/hr
X_1 = distance to the downstream thermocouple from the heat source in centimeters
X_2 = distance to the upstream thermocouple from the heat source in centimeters
T = time in seconds

Bloodworth, Page, and Cowley (1956) considered the thermoelectric method of approximating water use to be very valuable in determining the worth of various defoliants in reducing water use by cotton plants. Gale and Poljakoff-Mayber (1964) attempted to use the heat pulse method to study the effects of soil moisture stress on transpiration. When water potential in the soil decreased, heat pulse velocity increased in pine seedlings and decreased in sour orange. The increase in pine may have been due to a decrease in conducting area at high moisture stress. Wendt, Runkles, and Haas (1967) developed a regression equation designed to predict water use using the heat pulse method. A correlation coefficient of 0.919 was obtained for seedlings and 0.810 for one-year-old plants.

Effects of Meteorological Variables on Transpiration

Penman and Schofield (1951) emphasized the overriding importance of meteorological variables and not stomate size as affecting the rate and amount of transpiration occurring. Nutman (1941) stated that the details of the daily march of transpiration are due almost entirely to changes in incident solar radiation. He also reported that daily transpiration is affected somewhat by other factors such as relative humidity and air temperature. Wind velocity may also be important especially if relative humidity is high. A slight wind prevents a thick boundary layer of essentially 100 percent relative humidity building up around the stomata. Konis (1950) stated that the effect of radiation on transpiration may be attributable almost entirely to the increase in leaf temperature induced by the radiation. He further stated that rapid changes in transpiration rate with cloud cover are due to temperature changes in the leaves. Boersma and Cox (1967) measured transpiration rates under controlled conditions of soil water stress and soil temperature in

Trifolium repens. All other environmental factors such as relative humidity, air temperature, light intensity, and air speed were held constant. A significant interaction between soil moisture stress and soil temperature was observed. Wooley (1961) studied mechanisms by which wind influences transpiration in corn. These were mechanisms other than the removal of the boundary layer. None could account for as much as 1 percent of the transpiration from corn leaves.

Bloodworth, Page, and Cowley (1955) experimented with the effects of wind on heat pulse velocity. At high wind speeds they observed velocities up to 114 centimeters per hour. At low wind speeds the velocity averaged 76 centimeters per hour. With no wind, a rate of 38 centimeters per hour was observed.

STUDY AREA AND METHODS

The Study Site

The stand of quaking aspen where this study was conducted is located approximately 20 miles by highway northeast of Logan, Utah, on the Twin Creek subdrainage of the Logan River. The plots are at an elevation of approximately 7800 feet. They face south and lie on a slope of about 25 percent. Two plots were involved in the study. They lie adjacent to each other and each occupies approximately one-half acre. The exact dimensions of each plot are 100 feet by 250 feet. One is a control plot and the other received the anti-transpirant treatment.

Vegetation

A pure stand of mature quaking aspen constitutes the only overstory on the plots. All trees are of the same clone and so have the same genetic traits. The trees range in height from 40 to 60 feet and appear to be in relatively good health. Large amounts of bracken fern, some larkspur, mountain rue, and various grass species constitute the understory.

Soils and Geology

Soils on the site are deep and well developed. They are generally loamy in texture. The Twin Creek drainage is a glaciated valley, and numerous large rocks are in the soil profile.

Climate

The climate is typical of mountain country in the Intermountain Region. Summer days normally reach an extreme high of 80F and a low at night of about 55F. The winter night time temperatures reach below 0F and seldom are above 32F in the day. The area receives approximately 30 inches of annual precipitation and about 75 percent of this falls in the form of snow. Little rain falls during the summer months except during an occasional thunderstorm.

Application of PMA

The plot was sprayed by helicopter during the evening of June 13, 1968. PMA, at a concentration of 1×10^{-3} M, was used. Spray drift to the adjacent control plot was negligible due to accurate plot marking by helium balloons and calm winds during the hour of spraying. Many sheets of white paper were laid on the ground prior to the treatment so that some visible estimate could be made of the coverage of the spray. These papers were examined within about 10 minutes after the spraying operation and were found to be thoroughly spotted from the spray. The natural fluttering of the leaves of aspen facilitated the delivery of the PMA solution to the undersides of the leaves. Downdrafts of air from the helicopter blades caused visibly violent shaking of the trees. These same downdrafts seemed to force the spray down into the canopy.

Collection and Analysis of Stomate Data

Leaf stomate measurements were made using the method described by Sampson (1961). Silicone rubber monomer was mixed with the required amount of catalyst (the amount depends on the ambient air temperature) and was immediately spread over the underside of the leaf. The rubber usually dried in from three to four minutes and was then lifted off with the fingers. Applications were made on leaves that had been cut from the tree no more than one minute before application.

The dried impressions (which were essentially negatives of the leaf surface) were then taken to the laboratory where they were painted with a mixture of acetone and clear fingernail polish. When this dried, the positive, transparent impression was immediately mounted on a microscope slide, and stomate measurements were made under a microscope. Student's t-tests were used to compare the stomate data from treated trees with data from control trees.

Collection and Analysis of Heat Pulse Velocity Data

Heat pulse devices were installed on selected trees according to the method employed by Swanson (1968). Dominant and codominant trees were chosen because such trees were likely to show the effects of the PMA better than smaller trees. An area of bark about one inch by one-half inch was carefully removed inward to the cambium. A precisely machined template was placed over the bark cavity and three small holes were drilled one centimeter into the xylem of the tree. The top hole was drilled one centimeter above the center hole and the bottom one was drilled one-half centimeter below the center hole. A hypodermic needle was placed in the center hole and nichrome wire threaded into it. Nichrome was chosen as the heat conducting source because it is a high resistance wire. Each of the other two holes was fitted with a copper-constantan thermocouple which was designed for connection to a portable microvoltmeter. Leads from the heat source were attached in circuit to a six-volt storage battery through an instantaneous switch. An illustration of one set-up is shown in Figure 1.

The thermocouples were manually balanced in the internal

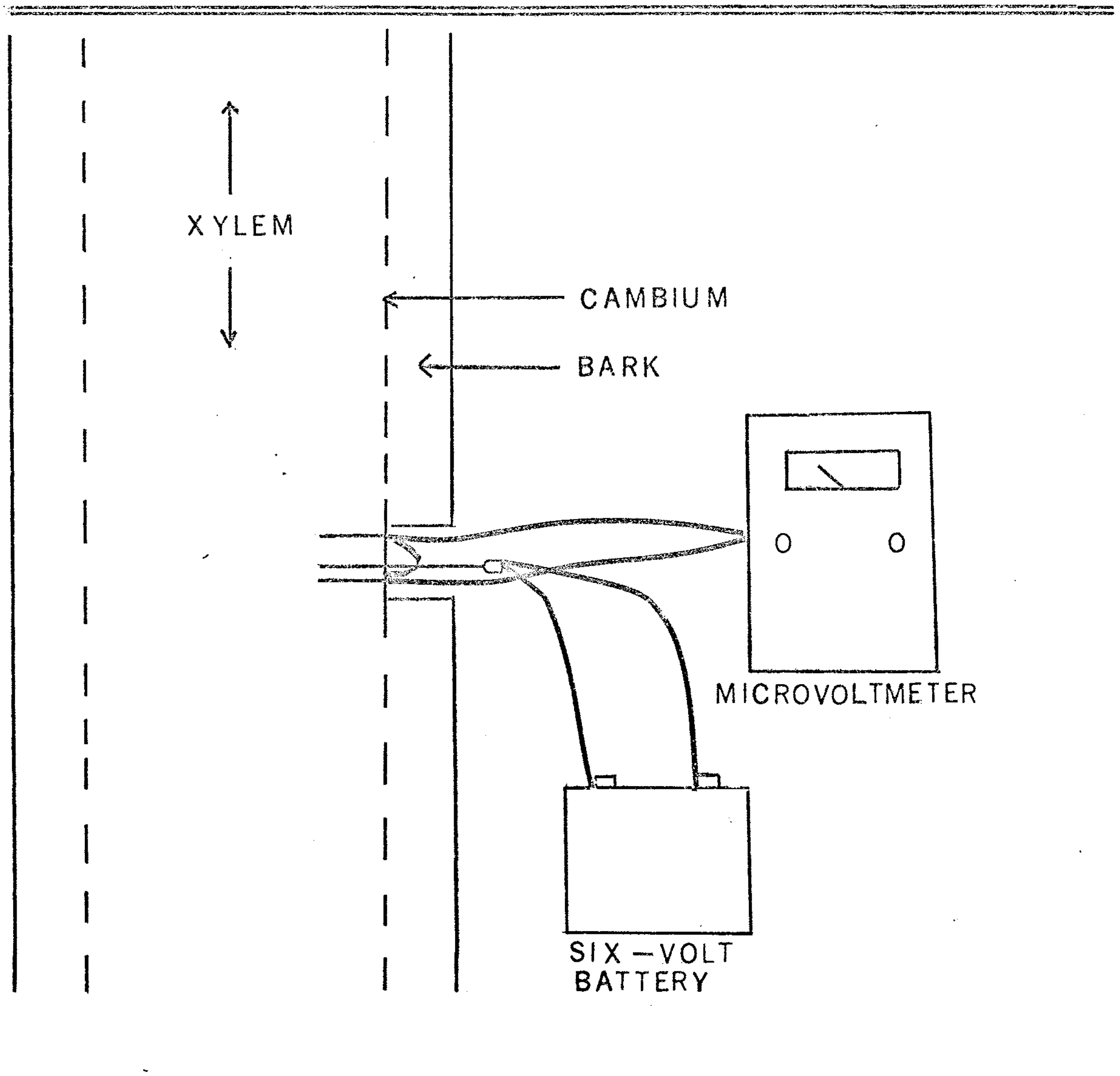


Figure 1. Schematic diagram of the heat pulse velocity apparatus used at Twin Creek.

system of the microvoltmeter. A stop-watch was held in one hand and the switch in the other. A heat pulse of two seconds duration was applied in the xylem, and the stop-watch was started at the instant heat was applied. By conduction, heat reached the bottom thermocouple first since it was closer to the heat source. This placed the thermocouples out of balance. The stop-watch was stopped when the thermocouples balanced again. This occurred when the heat pulse reached the downstream thermocouple by the combined processes of conduction and convection. The elapsed time is then entered into the formula $HPV = \frac{3600(X_1-X_2)}{2T}$. In our case this reduces to $HPV = \frac{900}{T}$. Swanson (1968) stated that the time (T) should not exceed 900 seconds or the heat will have dissipated to the atmosphere and the reading cannot be relied upon. The apparatus is shown in Figure 1.

Readings were taken on several trees on both the PMA and control plots. Velocities were recorded at approximately one-half hour intervals during days on which data were collected. This interval was used between readings in order to allow the heat applied each time to dissipate completely. Data were again analyzed using t-test group comparisons.

Measurements of Radial Growth

Measurements of diameter growth were obtained from the same trees used for heat pulse measurements. Increment cores were extracted from 12 trees in the fall of 1969. Two cores were taken from each of the trees. Six trees were chosen on each plot. Growth rings for the past five years were measured on each core. Values for 1969, 1966, and 1965 were for non-treatment years; and those for 1968 and 1967 were for treatment years. All measurements were made to the nearest one-half millimeter. Data from control trees were compared with those from trees treated with PMA by use of t-tests.

Measurement of Total Available Carbohydrates

In the fall of 1968 the current shoot growth was collected from numerous branches on several trees on both plots. Materials collected were oven dried at 70C. The twig tips were then ground to a powdery texture. This powder was then analyzed to determine the percent total available carbohydrates. Total available carbohydrates are those readily available for plant use.

Smith, Paulsen, and Raguse (1964) described the extraction process used. One gram of the sample was mixed with 50 ml of 0.2N H_2SO_4 and refluxed for 60 minutes in a boiling water bath. The solution was then filtered, and the filtrate was neutralized with NaOH and diluted with distilled water. Proteins were not removed as they often are in other extraction processes.

Shaffer and Somogyi (1965) described the final action. The reagent was prepared by dissolving 25 g each of anhydrous Na_2CO_3 and Rochelle salt in 500 ml of water. Then, 75 ml of a solution of 100 g $CuSO_4 \cdot 5H_2O$ was added. Next 20 g $NaHCO_3$ were added, dissolved, and then 5 g KI were added. Finally, 250 ml 0.1N KIO_3 were added. The determination was made in the following way. We pipeted 5 ml of solution containing 0.5 to 2.5 mg dextrose into a test tube. To this was added 5 ml reagent. A blank using 5 ml H_2O and 5 ml reagent was also prepared. The tubes were placed in a boiling water bath for 15 minutes. Then they were put in a cooling bath for four minutes. Added down the sides of each tube were 2 ml $KI\text{-}K_2C_2O_4$ solution and then 3 ml 2N H_2SO_4. They were mixed so that all Cu_2O dissolved. They were then put in a cooling bath for 5 minutes. This was titrated with 0.005N $Na_2S_2O_3$ using a starch indicator. Then the titrate of the test solution was subtracted from that of the blank. The amount of dextrose in 5 ml of solution was determined from a table in the handbook of the Association of Official Agricultural Chemists.

Data were then analyzed using the Student's t-test.

Collection of Meteorological Data

Air temperature and relative humidity were recorded in a nearby clearing with a hygrothermograph in a standard instrument shelter. Solar radiation and wind travel were measured on an actinograph and a cup anemometer atop a 60-foot tower located in the middle of the control plot. Soil moisture was monitored at 12 points in each plot at two-week intervals with a neutron moisture meter. A six-foot soil profile was sampled at one-foot intervals. Data were inserted into a multiple regression computer program and were used to predict heat pulse velocity.

RESULTS AND DISCUSSION

Heat Pulse Velocity

Since heat pulse velocity can be considered a reliable index to sap velocity and therefore transpiration rate, this method was used as the fundamental physiological measure of treatment effect. If PMA does collapse the stomates and reduce transpiration losses, then sap velocity in treated trees should be reduced. This hypothesis was tested with a simple t-test. Tests were made on 16 different days throughout the season. One test was run before the spraying operation and 15 following it. Readings used in the statistical analysis were gathered between 0900 and 1500 hours Mountain Standard Time on each day tested. Results are shown in Table 1.

Significance at any level is not common. However, an examination of the daily averages reveals a definite difference between the two treatments. The fact that there is an apparent difference but no statistical significance in many cases can be partly explained by sampling restrictions. We had only two microvoltmeters and two operators collecting data. To get reliable data it was necessary to read each

Table 1. Comparisons of heat pulse velocities on various dates.

Date (1968)	Average Heat Pulse Velocity Control Trees	PMA Trees	Degrees of Freedom	Statistical Significance[a]
	(cm/hr)			
June 11	9.3	6.4	4	3
June 14	11.0	5.9	3	3
June 18	13.6	6.1	5	2
June 19	13.6	5.4	5	3
June 21	13.5	7.2	5	3
June 24	14.0	5.4	5	1
June 25	12.7	5.9	4	1
July 2	12.2	8.2	8	3
July 3	12.6	8.9	7	3
July 17	10.8	6.9	9	2
July 18	10.9	9.1	7	3
Aug. 1	8.7	6.6	9	3
Aug. 16	7.2	1.5	4	1
Aug. 20	6.4	1.8	4	1
Aug. 21	4.6	2.7	7	2
Sept. 12	2.2	3.0	8	3

[a]The following code numbers are used to convey the significance level of each pair of heat pulse velocities: 1-significance at the .95 level, 2-significance between .90 and .95, and 3-significance below the .90 level.

tree about every half hour, so this limited the number of trees that could be metered within the half hour. We were able to collect data from only four or five trees on each plot. Since so few trees were used, the degrees of freedom used in the statistical analysis were necessarily low. There was often a great deal of variability in velocity from tree to tree within the same plot on a given day. This being the case, each observation does not contribute equally to the analysis especially when the numbers are squared. Control velocities on some days ranged from 5 cm/hour to 20 cm/hour.

Although significant statistical difference between the treatments did not occur every sampling day, it can be readily seen that there is a sizable difference in the mean transpiration rates of the two plots as indexed by the heat pulse velocity. In 15 out of 16 days, the average flow is less in the PMA trees than in the control trees. This difference persisted throughout the summer until in September when the weather began to turn cold and the transpirational demand of the trees on both plots was limited.

Several of the PMA trees were resprayed from the ground on August 10, 1968. No real effect from this treatment could be detected. This is probably due to the fact that heavy rains and cold temperatures accompanied this second treatment and reduced water use on both plots.

Phenylmercuric acetate leaves some stomates paralyzed in a partly open condition rather than closing all of them completely. This may account for the leveling out of the heat pulse velocity of the PMA trees at the end of the season even though the velocity of control trees continued to drop towards zero until leaf abscission occurred.

The relatively large differences that occurred until approximately July 15, (see Figure 2B) seem to indicate that the real effect of the PMA occurred during the first month after treatment. Following this period, the differences between treatments are noticeably less, although they persist. The pattern seen in August and early September may not be what would normally occur had dry, warm weather continued through September as it usually does in northern Utah.

Stomate Opening

Stomatal impressions were obtained from a few trees (usually about five) in each plot on several days during the season. Numerous samples were taken from each tree throughout each day. Only measurements of stomate width were used in the tests imposed on stomate data. This is because PMA affects only the width of the stoma and not the length of it. The same sampling problems associated with heat pulse velocity are involved here. A large number of trees could not be tested in one day and still obtain an adequate sample from all trees. Heat pulse data were collected on the same days as stomate data, so this reduced the time available for gathering impressions. Samples were taken throughout the day

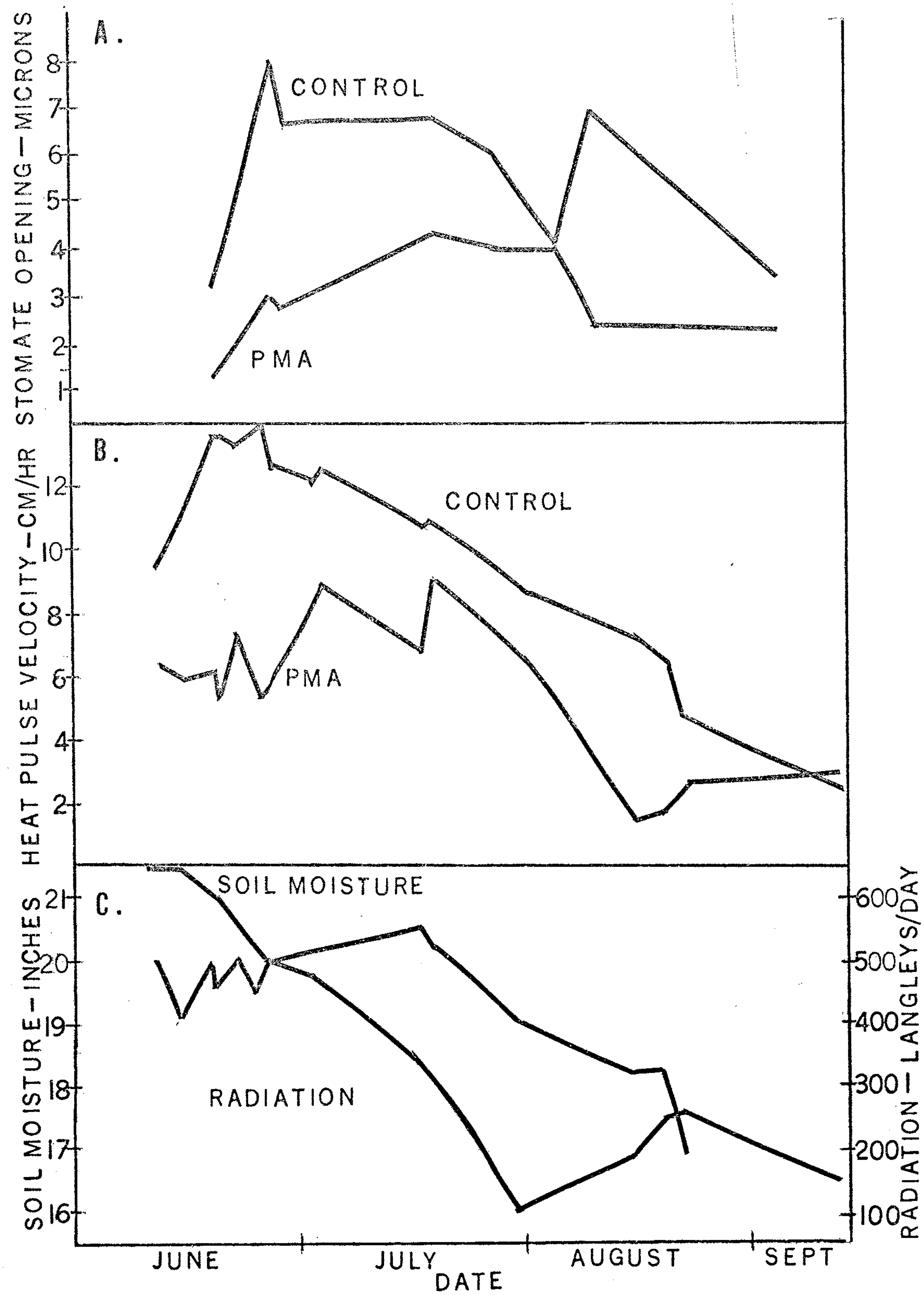


Figure 2. A. Control and heat pulse velocities on various days. B. Control and PMA stomate opening. C. Soil moisture and radiation throughout the season.

to obtain a true average from the changes that occur in stomate width during a daily cycle.

Table 2 and Figure 2A show the relationship between stomate widths for control and PMA trees. A comparison of heat pulse velocity and stomatal width in control trees is shown in Figure 3A. Figure 3B shows PMA heat pulse velocities in relation to stomate widths of PMA trees.

Table 2. Comparisons of average stomate widths on various dates.

Date (1968)	Average Stomate Width Control Trees	PMA Trees	Degrees of Freedom	Statistical Significance[a]
	(microns)			
June 18	3.24	1.23	9	1
June 25	8.00	2.96	3	3
June 26	6.68	2.80	9	1
July 17	6.88	4.28	13	1
July 25	6.07	3.98	3	3
July 31	4.07	3.97	5	3
Aug. 8	6.99	2.37	4	1
Aug. 29	3.50	2.34	3	3

[a]The following code numbers are used to convey the significance of each pair of stomate widths: 1-significance at the .95 level, 2-significance level between .90 and .95, 3-significance below the .90 level.

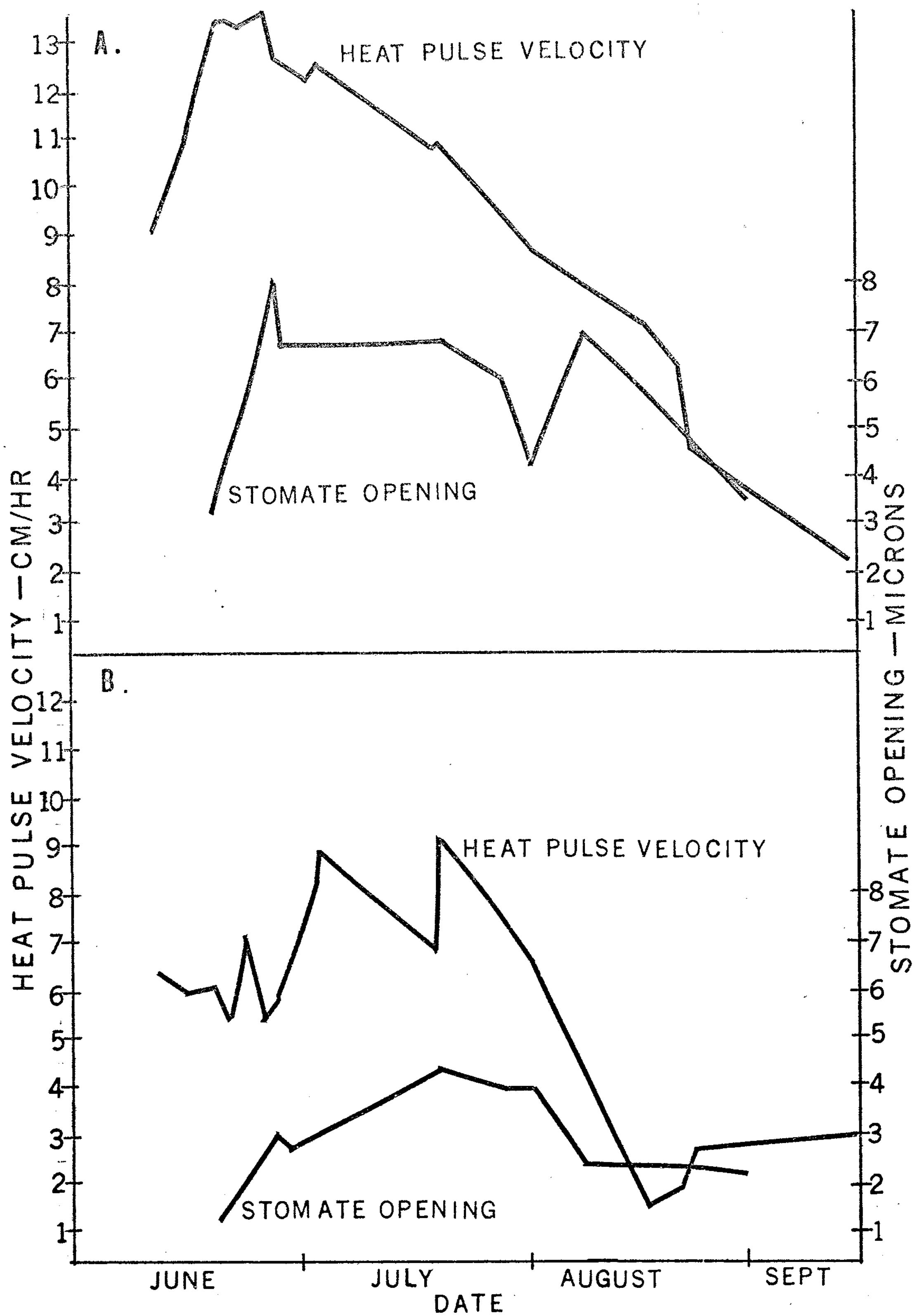


Figure 3. A. Comparison of heat pulse velocity and stomate opening for control trees. B. Comparison of heat pulse velocity and stomate opening for PMA trees.

Stomate width is an effective index for determining whether PMA reduces transpiration. Waggoner (1967) stated that transpiration is proportional to the area of the stoma when water is not limiting. A reduction in the width of opening will then reduce its area and hence transpiration. It can easily be seen that even in those cases where there was no significant difference between the treatments there was a numerical difference. This indicates that transpiration was reduced due to the addition of PMA.

Curves of stomate width over time graphically illustrate the difference mentioned above. Another important detail can be observed in Figure 2A. The PMA curve, although remaining below the control curve, gradually rises over time. This is probably an indication of the gradual loss of effectiveness of the PMA over the summer. Some lingering effect occurs throughout the entire study period because the curves never come together or cross. Figure 3B indicates a slight increase in heat pulse velocity until mid-August. This increase corresponds to the increase in stomate opening. The same correspondence is indicated late in the summer as both heat pulse velocity and stomate opening decrease.

An interesting aspect demonstrating the relationship of heat pulse velocity and stomate aperture of untreated trees is shown in Figure 3A. Velocity tends to decrease throughout the greater part of the summer but stomate width stays fairly uniform for an extended period of time. This can be explained by the fact that, as the summer progresses, soil moisture decreases. Stomate width may remain the same (transpirational demand remains the same) but the soil water deficit increases and so water is not as readily available to satisfy this demand as it was early in the season. Restated, the actual transpiration becomes less than the potential transpiration.

Radial Growth

Tests run on annual ring growth show a significant difference only in 1968, the second year of treatment with PMA. The t-tests were performed on the non-treatment years of 1965, 1966, and 1969 to see if there is normally any significant difference in ring width for trees growing on the two plots. A look at Table 3 and Figure 4 indicates that there was not, at least for those years tested. Assuming that ring width is normally not different, it can be said that treatment with PMA does affect growth to a certain extent.

Production of the annual ring involves utilization of carbohydrates produced in the previous year in addition to carbohydrates produced in the present year. The ring widths

Table 3. Annual ring width averages for control and PMA treated trees for treatment and non-treatment years.

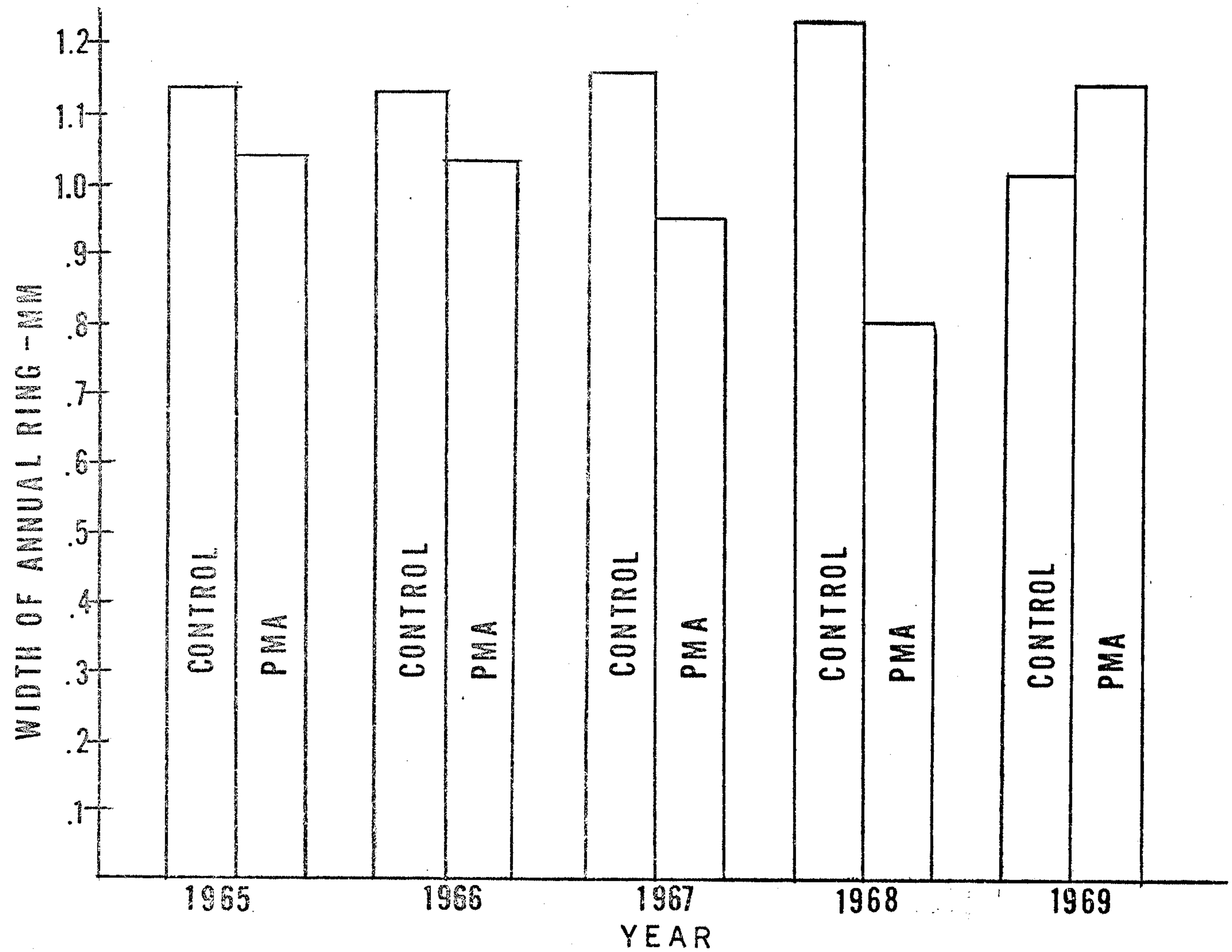
Year	Annual Ring Width Control Trees	PMA Trees	Degrees of Freedom	Statistical Significance[a]
	(mm)			
1965	1.14	1.04	22	3
1966	1.13	1.03	22	3
1967[b]	1.16	0.95	22	3
1968[b]	1.24	0.80	22	1
1969	1.02	1.14	22	3

[a]The following code numbers are used to convey the significance of each pair of ring widths: 1-significance at the .95 level, 2-significance level between .90 and .95, and 3-significance below the .90 level.

[b]This was a year in which trees were treated.

for 1967 indicate a distinct difference between treatments, although this difference is not significant. Since there was no treatment in 1966, stored carbohydrates would have been in normal abundance. Carbohydrates stored in 1967 may have been reduced by treatment and the second application of PMA in 1968 may account for the significant difference recorded in that year. The second application probably reduced production of carbohydrates in 1968. This, combined

Figure 4. Comparison of average annual ring width for control and PMA treated trees for various years.



with the reduced storage of carbohydrates in 1967, gave the significant difference noted. The absence of treatment in 1969 probably allowed adequate production of carbohydrates to overcome any deficit that occurred in 1969.

Total Available Carbohydrates

The total available carbohydrates (TAC) measured in milligrams per gram of sample on treated and control plots show no significant difference between the two plots at the 5 percent level. Six samples of each treatment were used in the analysis. The mean value of control TAC was 8.77 mg/g and 8.83 mg/g for the PMA trees. Since TAC is expressed as mg/g or as a percent, no inference can be made as to a quantitative difference between the plots. The reason that there is a significant difference in ring growth but not in TAC may be explained because of this. The actual biomass of new growth put on the PMA plot may be less than that put on the control plot during the growing season.

In conclusion, the application of phenylmercuric acetate does affect photosynthesis and growth of aspen, but the actual amount cannot be determined by the methods employed in this study.

Prediction of Heat Pulse Velocity

Development of a multiple regression equation that can be used to predict heat pulse velocity is important for two reasons: 1) to gain a better understanding of which climatic factors influence transpiration rate and to what extent they do so, and 2) to check the feasibility of another method of carrying out the same experiment tried in this study. Heat pulse velocity can be determined easily, because a simple equation can be used. Several trees can be metered for a pre-treatment period and their heat pulse velocities obtained. With these values, a prediction equation can be developed to predict these velocities under pre-treatment conditions. The equation can be developed using climatic variables. The trees can then be treated with an anti-transpirant. Heat pulse velocities under treatment conditions can be compared with predicted velocities under untreated conditions. This should give a good idea of the magnitude of change effected by the chemical.

Another method of employing regression analysis would be to calibrate the heat pulse velocities of one plot against another. Then one plot might be treated. Predicted control values from the regression equation could be compared with the actual velocities recorded after treatment. Such a method cannot be demonstrated in this paper because heat pulse velocities before treatment were not obtained for the PMA plot.

A multiple regression equation using climatic variables to predict heat pulse velocity was developed for the Twin Creek site. Five independent variables were used. These were solar radiation (cal/cm^2/min), wind (miles/hour), air temperature (degrees Fahrenheit), relative humidity, and soil moisture stress classes. The soil moisture stress classes were determined by dividing total soil moisture depletion for the summer into four equal classes. Each division was given a number of from one to four, depending on the amount of depletion that had occurred. All heat pulse velocities used in the development of the equation were from control trees growing at the Twin Creek study site. Values used were obtained at all times of the day and night so all conditions of radiation, temperature, humidity, and wind that occur at Twin Creek in the summer are represented. All parts of the growing season were sampled also, so soil moisture goes through relatively large changes. It is likely, however, that soil water is never unavailable for use by plants at Twin Creek.

A total of 412 observations was used in the analysis. The analysis was run as a stepwise multiple regression where the least important variable was deleted each time until a linear regression was obtained as the final equation. The variables used are as follows.

y = Heat pulse velocity (cm/hr)

X_1 = Radiation (cal/cm^2/min)

X_2 = Temperature (degrees Fahrenheit)

X_3 = Wind (miles/hour)

X_4 = Relative humidity (percent)

X_5 = Soil moisture (stress class)

The equations that resulted follow. The coefficient of determination (r^2) is also given.

Equation 1 - all variables present $r^2 = 0.30029$

$$y = 0.479 + 5.32X_1 + 0.131X_2 - 0.337X_3 - 0.0026X_4 - 0.846X_5$$

Equation 2 - humidity has been deleted $r^2 = 0.30028$

$$y = 0.71 + 5.32X_1 + 0.133X_2 - 0.336X_3 - 0.850X_5$$

Equation 3 - wind has been deleted $r^2 - 0.29518$

$$y = 0.66 + 4.62X_1 + 0.129X_2 - 0.908X_5$$

Equation 4 - air temperature has been deleted $r^2 = 0.27410$

$$y = 5.95 + 6.91X_1 - 0.799X_5$$

Equation 5 - soil moisture has been deleted $r^2 = 0.25137$

$$y = 3.80 + 7.24X_1$$

Radiation is clearly the variable contributing most to the regression. This can be said because radiation is the variable retained in the linear case. Humidity contributes least toward the regression since it is the first variable deleted in the step-wise process. Humidity is likely to be unimportant in this case because the humidity used in this case is the humidity of the general atmosphere. It is the humidity of the thin layer of air that surrounds the leaf that influences the rate of transpiration.

Even this is of little importance if a slight wind is blowing to keep the air moving around the leaves.

Wind was the second variable deleted. Wind movement is generally minimal at the site at Twin Creek because the plots are well protected. This may be the reason wind contributed so little (less than 1%) to the regression. Another factor that may have prevented a wider range of wind velocities is the fact that nearly three miles per hour of wind are required before the cups on the anemometer begin to turn. Therefore, small wind velocities and small velocity differences were not recorded.

Air temperature was the third parameter eliminated in the stepwise process. Perhaps a more sensitive temperature index would be leaf temperature. This temperature is more difficult to obtain than air temperature unless an instrument such as an infrared radiometer is available. The independent variables used in this study were chosen specifically because of the relative ease with which they could be measured.

Soil moisture was the fourth variable deleted. This factor made a fairly strong contribution to the regression but, perhaps, could have been more sensitive. If the soil bulk density, field capacity, and permanent wilting percentage had been known, it would have been possible to delineate some more real and fixed moisture stress classes. These might have been more meaningful in predicting the ability of the soil to supply water under different atmospheric demands. Soil water totals used were for a six-foot profile and little is known about the depth to which the trees on these plots are able to withdraw water.

One should probably expect radiation to contribute most to the regression. Its importance in the opening and closing of leaf stomata has been discussed earlier.

The solutions to the prediction equations indicate that, under conditions that yield a high heat pulse velocity, the predicted values are consistently one to two cm/hr low. When velocities are low the equations tend to predict high by from one to two cm/hr. If velocities are only moderately fast (five to seven cm/hr), the equations tend to predict actual values closely.

An examination of the r^2 values indicates that a great deal of variability is unexplained by the independent variables chosen. A coefficient of determination of 0.300 explains only 30 percent of the variability associated with heat pulse velocity. Width of stomate opening, natural stomatal resistances, CO_2 concentration, leaf temperature, soil temperature, and depth of rooting all have some effect on the rate of transpiration. None of these are included in these equations. Such factors are difficult to measure.

One thing shown by this type an analysis is that the process of transpiration is complex and not yet completely understood.

SUMMARY AND CONCLUSIONS

During the summers of 1967 and 1968 phenylmercuric acetate (PMA), an anti-transpirant chemical, was sprayed over a one-half acre plot of quaking aspen trees in northern Utah. The experiments reported in this thesis were designed to test the effectiveness of PMA in reducing transpiration and to evaluate its effect on the growth of the trees. A control plot,within the same clone of trees, was established adjacent to the treated plot. The study involved the measurement of heat pulse velocity (an index to transpiration), stomate width, diameter growth, and production of total available carbohydrates. Treated values were compared with control values for all these measurements.

The heat pulse method of estimating sap flow velocity was used to obtain estimates of transpiration rates. Silicone rubber impressions were used for the measurement of stomate aperture. Increment cores taken from treated and untreated trees were used for the determination of radial growth. Shoot growth of the current year was analyzed by chemical means to determine the total available carbohydrates.

Measurements of heat pulse velocities indicated distinct differences in velocities between control and treated trees.

These differences were not statistically significant in every case but the trees sprayed with PMA consistently had a slower rate of sap flow than control trees. Stomate widths in leaves of trees treated with PMA were distinctly narrower than the widths in control trees. These differences declined slightly as the season progressed and the effects of the chemical began to wear off. Radial growth differences among trees on the two plots were not statistically significant for any year tested except the treatment year of 1968. The treatment year of 1967 yielded a relatively large difference in ring width but it was not significant. No statistical difference was demonstrated in production of total available carbohydrates in mg/g of shoot growth. No determination was made of the total mass of shoot growth produced on control trees compared with sprayed trees, however.

A multiple regression equation was developed to predict heat pulse velocities of control trees. Independent variables considered were radiation, air temperature, relative humidity, wind, and soil moisture stress. The equation that was developed can be used to predict heat pulse velocity fairly accurately for moderate velocities. High velocities are under-estimated and low velocities are over-estimated.

The coefficient of determination (r^2) was low (0.300) and this indicates that many other separate and interrelated factors affect the transpiration rate of aspen. This solution also indicates that radiation, among the environmental variables examined, is the most important variable governing the rate of transpiration.

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VITA

Kenneth O. Higginbotham

Candidate for the Degree of

Master of Science

Thesis: The Effects of Phenylmercuric Acetate on the Transpiration and Physiology of Quaking Aspen.

Major Field: Forest Watershed Management

Biographical Information:

Personal Data: Born in Denver, Colorado, December 26, 1945, son of Kenneth O. and E. Jean Higginbotham; married Karen Brown June 3, 1966, two children--Kimberle, and Jeremy.

Education: Attended elementary school in Bozeman, Montana; graduated from Twin Falls Senior High, Twin Falls, Idaho in 1964; received the Bachelor of Science degree from Utah State University, with a major in forestry, in 1969; completed requirements for the Master of Science degree specializing in forest watershed management, at Utah State University in 1970.